Supplementary Material*

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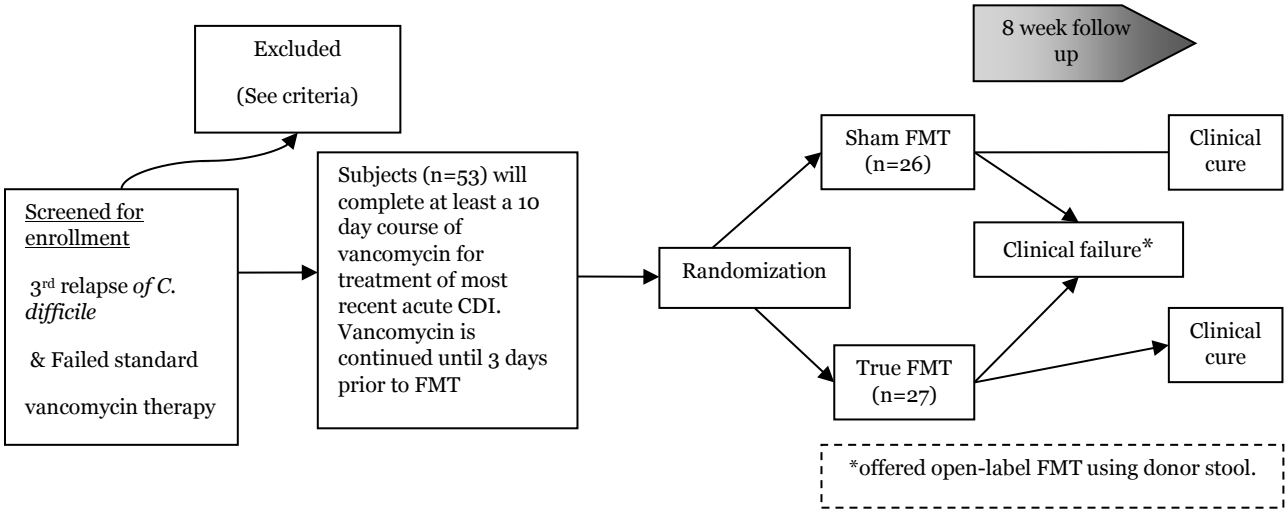
* This supplementary material was provided by the authors to give readers further details on their article. The material was reviewed but not copyedited.

APPENDIX A. STUDY PROTOCOL

I. Approach/Methods

This will be a randomized, double-blind, placebo (sham) controlled clinical trial to determine if FMT can prevent further recurrence in patients who have suffered at least a third relapse of *Clostridium difficile* infection (CDI) and who have previously been treated with oral vancomycin. Subjects will consist of adult outpatients referred to one of the study centers after 3 (or more) recurrences of CDI (defined as at least 3 unformed stools over 24 hours for 2 consecutive days and either positive stool testing for *C. difficile* or pseudomembranes on colonoscopy[6]). Subjects, who will have been treated with at least a 10 day course of vancomycin for the most recent acute infection, will then be randomized to receive FMT with donor stool administered at the time of colonoscopy or to receive sham FMT (colonoscopy and infusion with a suspension of the subject's own stool). After the procedure, subjects will be followed for 8 weeks for *C. difficile* recurrence. Sham-treated subjects who relapse during that period will be offered open-label treatment with FMT using donor stool. Subjects who received true FMT and subsequently relapse will be given the option of undergoing a second FMT using an alternate donor. We expect to conduct this study over a 24-month period of time.



Definitions

- *Clostridium difficile* infection: as per SHEA-IDSA guidelines[6], at least three unformed stools over 24 hours for two consecutive days and either positive stool testing (ELISA or PCR) for *C. difficile* toxins or pseudomembranes on colonoscopy
- Clinical Cure: resolution of diarrhea (i.e., fewer than three unformed stools for two consecutive days), with maintenance of resolution for the duration of the 8 week follow-up period and no further requirements for anti-infective therapy for C. difficile infection. Subjects who meet this definition will be considered cured regardless of results of follow-up stool testing for *C. difficile*.
- Clinical failure: persistence or development of diarrhea and the need for additional anti-infective therapy for CDI with or without positive stool testing (PCR) for *C. difficile.*

Inclusion criteria

1) Adult outpatients (age ≥18 and <75) referred to one of the study centers after suffering a third (or further) documented episode CDI and

2) who have failed to maintain CDI cure after standard therapy with oral vancomycin.

- Previous treatment with at least one course of tapered/pulse dose vancomycin as per SHEA-IDSA C difficile treatment guidelines or
- Inability to taper or stop vancomycin without developing diarrhea requiring anti-infective therapy.

Exclusion criteria

- Patients who are aged 75 years or greater.
- Patients who are pregnant
- Patients who are nursing
- Patients who are incarcerated
- Patients with cognitive impairment or severe neuropsychiatric co morbidities who are incapable of giving their own informed consent
- Patients who are immunocompromised specifically:
    - HIV infection (any CD4 count)
    - AIDS-defining diagnosis or CD4<200/mm_3
    - Inherited/primary immune disorders
    - Immunodeficient or Immunosuppressed due to medical condition/medication:
        - Current or recent (<3 most) treatment with anti-neoplastic agent
        - Current or recent (<3 mos) treatment with any immunosuppressant medications (including but not limited to monoclonal antibodies to B and T cells, anti-TNF agents, glucocorticoids, antimetabolites (azathioprine, 6-mercaptopurine), calcineurin inhibitors (tacrolimus, cyclosporine), mycophenolate mofetil). Subjects who are otherwise immunocompetent and have discontinued any immunosuppressant medications 3 or more months prior to enrollment may be eligible to enroll.
- Patients with a history of severe (anaphylactic) food allergy
- Patients who have previously undergone FMT
- Patients who are unwilling or unable to undergo colonoscopy
- Patients with untreated, in-situ colorectal cancer
- Patients with a history of inflammatory bowel disease (ulcerative colitis, Crohn's disease or microscopic colitis) or irritable bowel syndrome
- Unable to comply with protocol requirements
- Patients who are American Society of Anesthesiologists (ASA) Physical Status classification IV and V
- Patients with acute illness or fever on the day of planned FMT will be excluded (not undergo randomization or treatment with FMT) with the option of including that subject at a future date.

Randomization and blinding

Patients will be equally allocated to true FMT and sham FMT groups via block randomization by *C. difficile* positivity, stratified by study site. Specifically, within each site, patients who are *C. difficile* positive at baseline testing prior to FMT will be randomized separately from patients who are *C. difficile* PCR negative at baseline. Within each block, subjects will be randomized 1:1 to receive true vs. sham FMT. Randomization schedules for each study site will be generated in advance and maintained in password-protected files. Each patient will be assigned a unique identification number (UIN). Sequential UINs will be printed on cards and sealed in opaque envelopes. On the day of the procedure, a patient's eligibility will be confirmed during the study colonoscopy and the next available envelope within the block to which they belong will be opened. In the event the patient's study colonoscopy reveals ineligibility, the next envelope will not be opened and rather will go to the next fully eligible patient in that block. UIN and stool samples will be given to a non-blinded research assistant who will determine the appropriate treatment arm based on the randomization list. The UIN will be recorded on all study documents, in the database, and on the samples to preserve blinding of the physician/endoscopy-team and other research staff.

Power Analysis

FMT is expected to reduce the incidence of relapse from 50% to 10% at the end of the study (5-fold reduction). A Lan-DeMets alpha spending approach was taken, based on the O'Brien-Fleming bounds to allow for a single interim analysis setting alpha for that comparison to 0.3% with the remainder of 4.7% used for sample size analysis at the final analysis at the conclusion of the study, should the interim analysis not prove statistically significant at $p<.003$. Sample size of 48 at the time of analysis was estimated as necessary using Fisher's exact test (R version 2.10) to have >80% power at a two-sided alpha of 4.7%. Fisher's exact test chosen in anticipation of the possibility of predicted counts falling at or below 5. Sample size for recruitment was adjusted up to 53 in anticipation of an estimated 10% dropout.

II. Fecal Microbiota Transplantation Protocol

I. Donor selection and screening: Subjects may identify a donor (spouse/partner/intimate contact, household family member (adult-child, sibling), 1st degree family member outside the household (adult-child, sibling), other relative (aunt, uncle, cousin) or friend). For cases in which the subject does not have an available, qualified or willing donor, potential healthy volunteer donors will be recruited at each site and serve in a pool through an Institutional Review Board-approved process. Prospective donors will undergo a medical interview and physical exam as well as screening questionnaire and laboratory testing. For the blinded portion of the study, each donor will only be able to donate stool to one subject. For the open-ended portion of the study, an individual donor may donate stool for > 1 subject. Donors providing stool for a second FMT will undergo the necessary screening and testing on the same timelines as indicated below and in Appendix 6. This means that repeat donors my need to be retested or complete another DHQ.

A. Donor Interview: Potential donors will be interviewed and examined to determine the presence of systemic medical conditions which will preclude participation.

1. Established metabolic syndrome or early features suggestive of this [BMI >30 kg/m^2, waist: hip ratio >0.90 (male) and >0.85 (female); waist circumference >40 inches (male) or >35 inches (female); BP >135 systolic and >85 diastolic]
2. Known communicable disease
3. Systemic autoimmunity or atopic diseases
4. Chronic pain syndromes (for example: fibromyalgia, chronic fatigue)
5. Neurologic, neurodevelopmental or neurodegenerative disorders
6. Malignancy
7. Diarrheal disorder (IBS, IBD, celiac disease)
8. Use of antibiotics for any indication within the past 3 months

B. Donor Screening Questionnaire. Potential donors will be interviewed using a questionnaire based on the Donor History Questionnaire (DHQ) materials prepared by the AABB Donor History Task Force for use in screening blood donors. The DHQ is especially important to identify risks for diseases and conditions for which there are no laboratory tests, for which tests are not sensitive enough to detect infectious disease agents, and for which tests are unable to identify early stage or window period infections. Additional questions which are felt relevant to FMT, including recent use of antimicrobials, and history of relevant medical conditions have been added to this questionnaire (Appendix 1).
Our modified DHQ will be used to exclude donors with these and other risk factors:

1. High risk sexual behaviors (examples: sexual contact with anyone with HIV/AIDS or hepatitis, men who have sex with men, sex for drugs or money)
2. Known exposure to HIV or viral hepatitis within the previous 12 months.
3. Being held in a correctional facility for more than 72 hours in the last 12 months
4. Use of intravenous drugs or intranasal cocaine
5. Recent tattoo or body piercing
6. Recent transfusion, transplant or skin graft

7. Risk factors for variant Creutzfeldt-Jakob disease
Volunteer donors will complete the DHQ monthly, and those who develop any risk factors or will be excluded from further donation. Use of antibiotics will preclude donation for a period of 3 months.

C. Donor Laboratory Testing. HIV 1 & 2 testing will be performed within 2 weeks of donation for FMT. The other serologic and stool screening will be performed within one month of donation for FMT. (Appendix 2).

1. Stool:
    - *Clostridium difficile* toxin by PCR
    - Routine bacterial culture for enteric pathogens (E coli, Salmonella, Shigella, Yersinia, Campylobacter)
    - Culture for *Listeria monocytogenes* and *Vibrio* (*parahaemolyticus and cholerae*)
    - Fecal *Giardia* antigen
    - Fecal *Cryptosporidium* antigen
    - Acid-fast stain for *Cyclospora* and *Isospora*
    - Ova and parasites
    - Stool for Rotavirus via EIA

2. Blood:
    - HIV, type 1 and 2
    - HAV IgM
    - HBsAg, anti-HBc (both IgG and IgM), and anti-HBs.
    - HCV Ab
    - RPR

D. Day of Procedure: donor checklist. On the day of procedure, donor will be questioned regarding the following:

1. Fever, vomiting, diarrhea or other symptoms of infection within the last 30 days.
2. Ingestion of potential allergen where the recipient has a known allergy to the agent.
*Persons with history of severe food allergies are excluded from enrollment (see exclusion criteria above).

III. Subject eligibility and screening

A. Potential subjects will undergo a medical interview to determine eligibility for the study and a physical exam will be performed at the screening visit.

B. Serologic Testing will be done on all subjects to document baseline status prior to FMT including: HIV 1 & 2, Hepatitis A total, Hepatitis B surface Ag, surface Ab and core Ab, Hepatitis C Ab, and RPR.

C. Pre-treatment Medications

1. Subjects will complete at least a 10-day course of vancomycin for the most recently diagnosed acute CDI prior to undergoing FMT.
2. To prevent disease relapse while awaiting FMT, vancomycin will be continued by subjects up until 3 days prior to scheduled procedure.
3. The day before the procedure, the subject will be prepped with standard PEG bowel purge (4 liters polyethylene glycol solution).

D. Specimen collection

1. Subjects and donors will be supplied clean, sealable, color-coded containers for collection and transport of stool. They will be labeled with the subject (or donor) name, date of birth and date/time of collection.
2. Subjects will provide stool specimens at the following time points:
    a. between enrollment and 3 days prior to FMT (for microbiome analysis and *C. difficile* toxin testing)
    b. on the day of FMT (collected during the bowel purge for possible use in sham FMT only)
    c. 2 and 8 weeks after FMT (for microbiome analysis)
3. Donors will provide a fresh (<6 hour) specimen on the day of FMT (for possible use in FMT and for microbiome analysis)
4. All stool specimens will be kept refrigerated, transported on ice and used for FMT infusion or frozen at -70° Celsius within 6 hours of collection.
5. Pre and Post FMT urine samples will be collected for metabolic studies by the collaborating investigators. Fresh urine samples will be collected from donors and subjects at the following time points. These specimens will be frozen at -70° Celsius within 6 hours of collection.

- Donor urine (collected prior to FMT procedure only)
- Subject urine (collected ≥3 days pre-FMT and 2 weeks & 8 weeks post-FMT)

E. Female subjects
1. Females of childbearing potential will have a urine pregnancy test on the day of enrollment (to ensure eligibility) as well as on the day of planned FMT. Patients who are pregnant will be excluded.
2. Female subjects must not be and should not become pregnant nor breast-feed an infant while on his study. In order to reduce the risk of pregnancy, subject or her partner should use one or more of the acceptable methods of birth control listed below, regularly and consistently, while enrolled in this study. Acceptable methods of birth control (continuing throughout the study and for one month after the study) include:

- An approved oral contraceptive (birth control pill)
- Intra-uterine device (IUD)
- Hormone implants
- Contraceptive injection (Depo-Provera)
- Barrier methods (diaphragm with spermicidal gel or condoms)
- Transdermal contraceptives (birth control patch)
- Vaginal contraception ring (birth control ring)
- Sterilization (tubal ligation, hysterectomy or vasectomy)
- Abstinence

3. If subjects become pregnant or suspect that they are pregnant, or if they make someone pregnant, during this study, they will be instructed to immediately inform the study personnel. If subjects become pregnant or suspect that they are pregnant while on this study, a pregnancy test will be done. If pregnancy is confirmed, they will be withdrawn from the study if FMT therapy has not yet occurred. If FMT has occurred and conception occurs within the study period, the study physician will assist the patient in getting obstetrical care and will follow the progress of the pregnancy. The study physician will request access to subject and/or infant's medical records for up to at least eight weeks after delivery.

IV. Preparation of Stool for FMT Infusion (see Appendix 3)

A. Collection and handling

- Collected stool will be immediately refrigerated, transferred to the endoscopy facility on ice within a biohazard bag (but not frozen) and used within 6 hours of collection.

- Immediately prior to scheduled FMT procedure, the non-blinded research assistant (RA) will take possession of the donor and subject stool specimens. These will be labeled with UINs of donor and subject.
- Following randomization, either subject or donor stool material will be processed for FMT infusion.

B. Location & preparation of processing
- Stool will be processed in a designated area at each site. This area will not be accessed by the patient or investigators to avoid compromise of blinding.
- Universal precautions will be used during processing (gown, gloves, eye protection).
- Clean counter surface will be covered with a Chux® pad.
- After the FMT, all surfaces will be wiped with hospital-approved disinfectant solution.

C. Preparation materials
- 1 liter bottle of sterile, nonbacteriostatic normal saline
- Digital scale
- 60 cc disposable slip (catheter)-tip syringes
- Clean gauze
- polypropylene specimen containers

D. Preparation method
- Processing of stool will take place immediately before scheduled procedure.
- 500 cc of saline will be poured out of the 1 liter bottle and discarded.
- RA will suspend 100 grams of stool in 500 cc of sterile normal saline within the bottle.
- Bottle will be closed tightly and shaken vigorously for ~1 minute to homogenize the solution.
- This solution will then be filtered through a sieve to remove larger particulate matter.
- The fecal suspension will be drawn into five 60 cc slip (catheter) tip syringes for infusion.
- Syringes will be delivered to procedure area for administration during colonoscopy.
- The remaining stool specimens will be further processed and collected in polypropylene specimen containers for storage/shipping and eventual microbiome analysis.

V. FMT Procedure.

A. Randomization and preparation

1. Upon arrival for the procedure, subjects will be randomized by an un-blinded research assistant. Subjects will be randomized into one of 2 treatment arms in a 1:1 fashion to either receive true FMT using donor stool or sham FMT using their own stool specimen collected during the bowel prep. Subjects and physician/endoscopy-team will be blinded to the treatment.

2. The un-blinded research assistant will suspend 100 grams of fresh (<6 hour) donor or subject stool in 500 mL of sterile, nonbacteriostatic normal saline. The stool-saline suspension will be drawn into five 60 cc catheter tip syringes for infusion at colonoscopy. For detailed stool processing protocol see Appendix 3.

B. Colonoscopy and infusion.

1. Procedure will be performed within the endoscopy units of the study centers (Montefiore Medical Center; The Miriam Hospital).

2. 1-2 hours before colonoscopy, the subject will take 2 loperamide tablets to aid in retention of administered donor stool.

3. Colonoscopy will be performed and depth reached will be documented (typically the cecum or terminal ileum).

4. The endoscopist will administer 300 mL of the fecal suspension in aliquots of 60 mL, through the colonoscope starting at the most proximal point of insertion (terminal ileum or cecum) up through the proximal transverse colon.
5. The subject is encouraged to retain stool for as long as possible (optimally 1 hour). If unable to retain stool for this period, time of first BM post-procedure will be documented.

VI. Follow up

- Subjects will be encouraged to contact the clinical team if they experience recurrence of diarrhea so that stool can be tested for *C. difficile* toxins A & B.
- Subjects will be provided with a thermometer for daily recording of temperature (oral) and a diary card to record adverse events (AEs) solicited events for 7 days and unsolicited events for 30 days post- transplant.
- Subjects will be contacted via telephone by a study representative 2 and 7 days post FMT and then biweekly for a total of 8 weeks.
- Regardless of symptoms, all subjects will be seen in the clinic for follow up visit at 2 weeks and 8 weeks post-treatment. Subjects will submit stool specimens at time of 2 week and 8-week post-FMT visits for *C. difficile* toxin testing and microbiome analysis.
- Subjects will be contacted via telephone by a study representative 6 months after the last treatment to record any SAEs, new medical conditions/diagnoses or changes in medical conditions/medications since last study contact.

A. Efficacy Endpoints

- The primary efficacy endpoint will be the rate of "Clinical Cure" (defined above) in the intention-to-treat populations at the end of the 8-week period following FMT or at the time of early withdrawal from the study.
- The secondary efficacy endpoint will be "Clinical Failure" (defined above) in the intention-to-treat population during the 8-week period after FMT. Upon clinical failure, subject's treatment will be unblinded and those who received sham FMT may chose to receive open label FMT* using donor stool. Subjects, who received true FMT and develop clinical failure, may chose to undergo a second FMT* using an alternate donor.

Subjects who experience clinical failure after FMT (true FMT or sham) may, at the discretion of the investigator, be offered repeat FMT via unsedated sigmoidoscopy (instead of colonoscopy) for the open label portion of the study

B. Safety Endpoints

Safety will be evaluated through a review of summaries of:

- SAEs
- AEs
- Deaths
- New medical conditions/diagnoses or changes in medical conditions/medications at 6 month follow up contact

C. Subjects who relapse

1. At time of clinical failure, the subject's treatment arm will be unblinded. Subjects who experience clinical failure (relapse) within the 8-week period after FMT will be treated with a 10-day course of oral vancomycin.

- Subjects who received true FMT may choose to undergo a second FMT using an alternate donor. This second FMT will be given after the subject has completed at least a 10-day course of oral vancomycin.

- Subjects who received sham FMT may choose to undergo a second FMT using actual donor stool. This second FMT will be given after the subject has completed at least a 10-day course of oral vancomycin.
- Subjects may chose not to receive further FMT, but may continue to be treated at the study center in accordance with the current standard of care. The will continue to be followed for closely for AEs with telephone calls and study visits as per protocol.
- Alternatively, subjects who chose not to receive further FMT may decide to be treated at another facility of their choosing. They will still be followed as per protocol through the 8 week post FMT period and asked to receive a 6 month follow-up telephone call to assess for late SAEs or changes in medical conditions/diagnoses or medications.

Subjects who chose to undergo a second open-label FMT within 8 weeks post first FMT will have the identical procedure, including follow up, safety monitoring and microbiome analysis as per the schedule in Appendix 4 and 5. At discretion of the investigator, repeat FMT may be offered via sigmoidoscopy (instead of colonoscopy) for the open label portion of the study. This is to eliminate the additional risks associated with colonoscopy in subjects who may not have a clear indication for another colonoscopy Subjects who relapse within 8 weeks will be censored from the primary safety analysis at the time of treatment of relapse.

2. Subjects who relapse between 8 weeks and 6 months post FMT will be treated outside the study protocol in accordance with the current standard of care either at the study center or by an alternate facility/physician of their choosing. Data will not be collected prospectively in these subjects; however they will receive the 6-month follow up phone call for solicitation of SAEs and changes in medical conditions/diagnoses/medications.

E. Withdrawal of subjects

The study report will include all reasons for subject withdrawal.

Subjects who withdrawal from the study will be characterized as follows:

1. Subjects who withdrawal after enrollment (initial visit/informed consent) but <u>before randomization</u> (day of FMT) will not undergo FMT and, thus, cannot be included in statistical analysis.
2. Subjects who receive FMT and are then lost to follow up will be included in data analysis based on last available data point. Every reasonable effort will be made to contact these subjects to ensure they are receiving appropriate follow up care with documentation of any SAEs/AEs or changes in medical conditions/diagnoses or medications.
3. Subjects withdrawn because of SAE/AE or at the discretion of the physician-investigator will continue to receive treatment (outside the study protocol) in accordance with current standard of care. Subjects having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the investigator. Those who have undergone randomization/FMT will be included in data analysis based on last available data point.
4. Subjects who are discovered to be ineligible during the study colonoscopy (for previously unrecognized inflammatory bowel disease or in-situ colorectal cancer) will not receive FMT and will be excluded from the study. No data or safety analysis of these patients will be performed. These subjects (if already randomized) will be replaced.

Subjects who withdraw from the study will not be replaced. We anticipate 10% of subjects will drop out of the study. Sample size was adjusted upward to provide adequate net sample size (see power analysis).

VII. Results

<u>A. Data Collection (recorded in study database)</u>:

1. Demographics for subjects and donors: age, gender, race/ethnicity.
2. CDI inciting antibiotic (if any) and indication for use

3. Duration of C. *difficile* infection: number of months since initial diagnosis
4. Number of C. *difficile* recurrences
5. Total duration on metronidazole (# weeks)
6. Total duration on oral vancomycin (# weeks)
7. Other treatments received: (Intravenous Immunoglobulin, probiotics (specified), rifaximin, Fidaxomicin, nitazoxanide)
8. Screening laboratory test results (Subject & Donor)
9. Stool for C. *difficile* toxin at weeks 2 and 8 as well as additional time points as dictated by symptoms
10. Findings at colonoscopy (e.g. pseudomembranes, diverticula, colitis); extent reached by colonoscopy
11. Time to first BM after FMT procedure (if < 1 hour)
12. Pathology results (if applicable; random biopsies not routinely obtained for this study)
13. Symptoms at follow up (stool form/frequency, presence of abdominal pain, fevers, subjective well being).
14. Endpoints reached and, in instance of clinical failure, time post-FMT at which this occurred
15. Adverse events
16. New conditions or medical diagnoses or changes in medical conditions/medications elicited at 6 month follow up contact.

B. Adverse Events

We will specifically document:

- Complications related to the colonoscopy (sedation related, perforation, bleeding)
- Complications related to FMT (infection, inflammatory or allergic reaction)
- Solicited and unsolicited AEs, including fever, will be recorded by subjects on a diary card which will be distributed to the subject on the day of FMT.
- Development of new symptoms/diagnoses (irritable bowel syndrome, inflammatory bowel disease, autoimmune disorder, neurologic disorder) which may be related or unrelated to FMT will be elicited at the 6-month follow up telephone call and documented.

1. Subject reporting of AEs:

Subjects will have a diary card (Appendix 7) to record solicited adverse events (AEs) and fever for 7 days post-transplant and unsolicited AEs for 1 month post-transplant. Subjects will be given instructions for completing this card on the day of enrollment. This card will then be distributed to the subjects on the day of FMT procedure. There will be space on this card to record the intensity and duration of these AEs along with any actions taken. Patients will be called 2 days post FMT and reminded to complete the diary card. Diary cards will be collected and reviewed at 2 and 8-week post-FMT visits (Appendix 5). Subjects will be given a thermometer to record daily temperature (orally) in this diary at the same time each day. In cases of fever, subjects will be instructed to record the highest daily temperature. The following AEs will be solicited along with the intensity of each:

- Fever
  - Mild 37.7-38.6°C
  - Moderate 38.7-39.3°C
  - Severe 39.4-40.5°C
  - Potentially Life Threatening >40.5°C
- Chills
  - Mild: no or minimal interference with usual social and functional activities
  - Moderate: greater than minimal interference with usual social and functional activities
  - Severe: inability to perform usual social and functional activities

- Fatigue/Malaise
    - Mild: no or minimal interference with usual social and functional activities
    - Moderate: greater than minimal interference with usual social and functional activities
    - Severe: inability to perform usual social and functional activities
    - Potentially Life Threatening: Incapacitating fatigue/malaise symptoms causing inability to perfume basic self-care functions.
- Anorexia (loss of appetite)
    - Mild: Loss of appetite without decreased oral intake
    - Moderate: Loss of appetite with decreased oral intake without significant weight loss
    - Severe: Loss of appetite with decreased oral intake associated with significant weight loss
    - Potentially Life Threatening: Life threatening consequences or aggressive intervention indicated (TPN or tube feeding)
- Abdominal pain
    - Mild: Pain causing no or minimal interference with usual social and functional activities.
    - Moderate: Pain causing greater than minimal interference with usual social and functional activities.
    - Severe: Pain causing no inability to perform usual social and functional activities.
    - Potentially Life Threatening: Disabling pain causing inability to perform basic self-care functions OR hospitalization (other than an emergency room visit) indicated.
- Bloating
    - Mild: no or minimal interference with usual social and functional activities
    - Moderate: greater than minimal interference with usual social and functional activities
    - Severe: inability to perform usual social and functional activities
- Gas/Flatulence
    - Mild: no or minimal interference with usual social and functional activities
    - Moderate: greater than minimal interference with usual social and functional activities
    - Severe: inability to perform usual social and functional activities
- Constipation
    - Mild: irregularity of BMs not requiring dietary modification, laxative or enema.
    - Moderate: persistent constipation requiring regular use of dietary modifications, laxatives or enemas.
    - Severe: Obstipation with manual evacuation indicated
    - Potentially Life Threatening: Life threatening consequences (e.g. obstruction)
- Diarrhea
    - Mild: Transient or intermittent episodes of unformed stools OR increase of ≤3 stools over baseline per 24-hour period
    - Moderate: persistent episodes of unformed to watery stools OR increase of 4-6 stools over baseline per 24-hour period
    - Severe: Bloody diarrhea OR increase of ≥7 stools per 24-hour period OR IV fluid replacement indicated
    - Potentially Life Threatening: Life threatening consequences (e.g. hypotensive shock)

- Nausea
    - Mild: Transient (<24 hours) or intermittent nausea with no or minimal interference with oral intake.
    - Moderate: Persistent nausea resulting in decreased oral intake for 24-48 hours
    - Severe: Persistent nausea resulting in minimal oral intake for >48 hours OR aggressive rehydration indicated (IV fluids).
    - Potentially Life Threatening: Life threatening consequences (e.g. hypotensive shock)
- Vomiting
    - Mild: Transient or intermittent vomiting with no or minimal interference with oral intake.
    - Moderate: Frequent episodes of vomiting with no or mild dehydration.
    - Severe: Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated (IV fluids).
    - Potentially Life Threatening: Life threatening consequences (e.g. hypotensive shock)

VIII. Adverse Event Reporting

A. Definitions

1. Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial subject administered FMT that does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a FMT, whether or not related to the FMT.

• AEs may be new events or may be pre-existing conditions that have become aggravated or have worsened in severity or frequency.

• AEs may be clinically significant changes from baseline in physical examination, laboratory tests, or other diagnostic investigation (e.g., laboratory results, x-ray findings).

Pregnancy is not an AE; however, if a female subject becomes pregnant during the conduct of the study, the Investigator will notify sponsor who is responsible for notifying the FDA.

2. Serious Adverse Event (SAE)

A serious adverse event is any adverse experience occurring during or after FMT that results in any of the following outcomes:

• Death;

• Life-threatening experience;

Note: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

• Requires inpatient hospitalization or prolongation of existing hospitalization.

Note: Adverse events requiring hospital admissions that are less than 24 hours in duration do not meet this criterion. A scheduled hospitalization for a pre-existing condition that has not worsened during participation in the study does not meet this criterion. Pre-planned hospitalizations for an elective medical/surgical procedure or routine check-ups do not meet this criterion;

• Results in persistent or significant disability or incapacity;

• Is a congenital anomaly or birth defect;

• Is considered to be an important medical event.

Note: Important medical events are those that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

3. Planned Hospitalization
A hospitalization planned prior to FMT is to be considered a therapeutic intervention and not the result of a new SAE. If the planned hospitalization or procedure is executed as planned, it will be recorded in the subject's medical history or procedures. However, if the event/condition worsens during the trial, it must be reported as an AE.

4. *Adverse reaction*

An adverse reaction means any adverse event caused by FMT. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that FMT caused the event.

5. *Suspected Adverse Reaction*
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that FMT caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between FMT and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

6. *Unexpected*
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

B. Monitoring
1. Monitoring of Adverse Events
Each subject will be monitored for the occurrence of AEs, including SAEs, beginning immediately after FMT. Each subject will be followed for safety monitoring according to Appendix 5: Schedule of Events.

- Subjects will have a diary card to record solicited adverse events (AEs) and fever for 7 days post transplant and unsolicited AEs for approximately 1 month post transplant.
- Subjects will be questioned at each follow up time-point (Appendix 5) regarding stool form/frequency, presence of abdominal pain, fevers and subjective well being and/or examined (at 2 and 8 week follow up visits) by the investigator or a qualified designee for evidence of AEs. The questioning of subjects with regard to the possible occurrence of adverse events will be generalized such as, "How have you been feeling since your last visit?"
- Subjects having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the investigator.
- AEs, actions taken as a result of AEs, and follow-up results must be recorded in the Case Report Forms as well as in the subject's source documentation. Follow-up laboratory results should be filed with the subject's source documentation.
- Subjects will receive a follow up phone call 6 months post transplant to record any SAEs, new medical conditions/diagnoses or changes in conditions or diagnoses since last study contact.

For all SAEs and AEs that require the subject to be discontinued from the trial, relevant clinical assessments and laboratory tests will be repeated as clinically appropriate, until final resolution or stabilization of the event(s).

C. Assessment of Adverse Events

1. Assessment of Severity

The severity of AEs will be assessed according to the following definitions:

•Mild: the AE is noticeable to the patient and/or the Investigator, but does not interfere with routine activity.

•Moderate: the AE interferes with routine activity, but responds to symptomatic therapy or rest.

•Severe: the AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy.

2. Assessment of Causality

The Investigator must assess the relationship of any AE (including SAEs) to FMT, as related or not related, based on clinical judgment and using all available information, and may include consideration of the following factors:

• Possible alternative causes of the AE, including the disease under treatment, pre-existing conditions, concomitant use of other drugs, and presence of environmental or genetic factors.

• The temporal association between FMT exposure and onset of the AE.

• Whether the manifestations of the AE are consistent with known actions or theoretical toxicity of FMT.

The causal relationship between FMT and the AE will be assessed using one of the following categories:

Not Related: An AE is not associated with FMT if:

• Temporal relationship is lacking (e.g., the event did not occur within a reasonable time frame following administration of FMT); or

• Other causative factors more likely explain the event (e.g. pre-existing condition, other concomitant treatments);

Related: An AE is attributed to FMT if:

• There is a positive temporal relationship (e.g., the event occurred within a reasonable time frame following FMT); and

• The AE is more likely explained by FMT than by another cause

D. Reporting Safety Observations by the Investigator to the Sponsor

1. Reporting of Nonserious AEs

All AEs, regardless of seriousness, severity, or causal relationship to FMT, will be recorded on the AE page of the subject case report form (sCRF).

2. Reporting of FMT Exposure during Pregnancy

If a female subject or the female partner of a male subject becomes pregnant during the course of study, the Investigator must report this to the sponsor within 24 hours of becoming aware of the pregnancy. The Investigator is required to follow up on the pregnancy until it has completed. The outcome of the pregnancy and the status of the newborn (if applicable) will be reported within 24 hours of becoming aware. If the female partner of a male subject becomes pregnant, the Investigator must obtain consent to collect pregnancy information (including the status of the newborn, if applicable).

3. Reporting of Safety Observations by the Investigator

Any occurrence of the following events or outcomes in a subject in the trial must be reported expeditiously by the investigator or qualified designee to the local IRB and to the sponsor who will be responsible for reporting the event to the FDA.

1. SAE
2. Death of a subject

The investigator is to report any safety observations from the list above to the sponsor within 24 hours of becoming aware of the event. Any observation that is also an AE will be recorded on the sCRF along with

any actions taken. If not all information is available at the time of initial report, follow up SAE reports will be completed and submitted.

The investigator is required to follow SAEs until resolution regardless of whether the subjects are still participating in the study. Resolution is defined as:

- Resolved with or without residual effects
- Return to baseline for a pre-existing condition
- Fatal outcome; if autopsy is performed, the autopsy report must be provided to the sponsor.

4. Protocol-Specific Exceptions to (Serious) Adverse Event Reporting

In this trial, each suspected clinical endpoint event is to be reported in the sCRF. A suspected clinical endpoint event, regardless of when the event occurs, is not to be reported as an AE or SAE in the eCRF or reported in an expedited manner as an SAE.

The suspected clinical endpoint event includes:

Recurrent CDAD: The subject meets all of the following:

• Initial response to therapy with cure of CDI at end of treatment (vancomycin) pre-FMT; and

• A minimum of three unformed bowel movements over a 24-hour period

and

• A positive result for *C. difficile* toxin by EIA or PCR.

E. Monitoring the study database and submitting safety reports.

The sponsor will notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. The sponsor will notify the FDA and all participating investigators in an IND safety report of potentially serious risks from this clinical trial as soon as possible, but no later than 15 calendar days after the sponsor receives the safety information and determines that the information qualifies for reporting. *Participating investigators* include all investigators enrolling subjects under the sponsor's IND. In addition, the sponsor will identify in each IND safety report, all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and will analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant. The sponsor will evaluate a suspected adverse reaction in the context of other related reports or adverse events. Sponsor will periodically review and analyze the entire safety database (see data and safety monitoring plan), for IND safety reporting purposes, and also to update investigator brochures with new safety information. An IND safety report will be submitted when any of the following criteria are met:

a. Serious and unexpected suspected adverse reaction

b. Findings from other sources

The sponsor will also report expeditiously any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings that suggest a significant risk in humans exposed to FMT.

c. Increased occurrence of serious suspected adverse reactions

The sponsor will report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

F. Unblinding

The blind will be broken for serious and unexpected adverse events that meet the criteria for reporting. Knowledge of the treatment received is necessary for interpreting the event, may be essential for the medical management of the subject, and may provide critical safety information that could have implications for the ongoing conduct of the trial.

G. Halting Rules

Specific safety findings will result in temporarily suspending enrollment until a safety review is convened, the objective of which is a decision as to whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group or for the entire study) is another potential outcome of a safety review.
Subsequent review of serious, unexpected, and related AEs by the DSMB, IRB, the sponsor, or the FDA or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of FMT at a site. The FDA and study sponsor(s) retain the authority to suspend additional enrollment and study interventions/administration of FMT for the entire study, as applicable.

Findings that will trigger a safety review are

- Death
- Transmission of an infection from donor to subject via FMT
- If more than 10% of subjects experience the same Grade 3 Adverse Event
- If one or more subjects experiences a serious, unexpected adverse event
- Increased frequency of events (specifically, new diagnoses of inflammatory bowel disease in > 2 FMT treated subjects).

For individual subjects enrolled in the study: any subject who has an SAE related to FMT will be ineligible for a second FMT during the course of the study.

FDA/CBER will be notified by phone or fax within 48 hours if the study is halted for review.

IX. Data analysis

Descriptive statistics will be computed periodically and reported in aggregate as part of the study monitoring process. Analysis by treatment arm will occur once all data have been entered and cleaned and the database is locked. Baseline patient characteristics will be compared between groups to assess the adequacy of the randomization. Characteristics that differ markedly between groups will be considered as covariates in multivariable regression analyses. An intention-to-treat analysis will be conducted to estimate the efficacy of FMT for preventing *C. difficile* colitis relapse within 8 weeks post-therapy. Efficacy will be summarized by risk differences and odds ratios, along with 95.3% confidence intervals (CIs), and Fisher's exact test will be used to determine statistical significance at the two-sided 4.7% level (adjusting for one interim analysis). Odds ratios adjusted for study site, blocking, and any imbalanced baseline factors will be estimated by multivariable logistic regression. The impact of missing outcome data will be examined by multiple imputation and best case/worst case imputation. Additionally, a per-protocol analysis will be performed in the event of protocol deviations. A secondary analysis will focus on patients who receive the sham therapy, relapse, and then receive donor stool. The proportion of subjects who relapse again following the treatment will be calculated and compared by patient characteristics using a Chi-square test or Fisher's exact test when cell counts are low. Adverse events will be tabulated by study arm using Fisher's exact test. Proportions and 95% CIs will be computed. If zero events are observed during follow-up, an exact upper 95% CI will be computed as an upper bound on the plausible event proportion.

Banking of stool specimens for microbiome analysis (collaborative study)

Two samples from each stool specimen will be banked. One is for shipping to the collaborating investigator for microbiome studies. The other is to remain at the study facility for internal banking until the conclusion of the study (at least until the 6 month telephone contact with the last study patient).

The following samples of stool will be kept on ice and frozen at -70 Celsius within 6 hours of collection.

- Donor stool (collected day of procedure only)
- Subject stool (collected ≥3 days prior to FMT and 2 weeks & 8 weeks post-FMT)

Samples will be packed in dry ice and shipped to the laboratory of Drs. Alexander Khoruts and Michael Sadowsky at the University of Minnesota, Minneapolis where microbiome analysis will be performed. Collection/storage and shipping techniques detailed in Appendix 4.

Banking of urine specimens for metabolic analysis (collaborative study)
Two samples from each urine specimen will be banked. One is for shipping to the collaborating investigator. The other is to remain at the study facility for internal banking until the conclusion of the study (at least until the 6 month telephone contact with the last study patient).

The following samples of urine will be kept on ice and froze at -70 Celsius within 6 hours of collection.

- Donor urine (collected day of procedure only)
- Subject urine (collected ≥3 days prior to FMT and 2 weeks & 8 weeks post-FMT)

Potential Limitations

1. Difficulty recruiting enough patients willing to undergo FMT

- The investigators have built referral practices for FMT and expect to continue to see at least 5-6 new patients per month at each study center. Each of these patients will be screened for enrollment.

2. Some patients will refuse to be involved in the study because of the potential to receive sham treatment.

- This will be minimized by offering sham-treated subjects who relapse with CDI true FMT using donor stool. If this factor proves to be a significant barrier to recruitment, we will modify study protocol to 2:1 randomization so the chances of receiving true FMT are greater than the chances of receiving sham FMT.

3. Subject cooperation. Subjects and donors will bring samples of their own stool. Our results could be compromised by human error in the stool collection or labeling process.

- This will be minimized by providing labeled, color-coded stool collection materials and clear instructions to subjects and donors. Subjects and donors will have ample contact with the study coordinator so that all questions can be answered and follow up maintained.

X. Risks to Human Subjects

1. IRB approval for publication of preliminary data was obtained; Local IRB approval from each study center will be obtained. IND from FDA required before IRB approval of the clinical trial can be obtained.
2. Subjects will be given informed consent using the standard consent process for colonoscopy. Additional theoretical risk for transmission of infectious agents, allergens, and other diseases and conditions will also be discussed.
3. Donor will be given informed consent and will be tested for most common infectious agents. Individuals with communicable disorders or a history of high-risk behaviors will not be permitted to donate. Patient privacy rules will be applied to donor screening and testing.
4. Protections against risk are provided in detail below.

Human Subjects Involvement, Characteristics and Design

A sample size of 53 patients will be recruited. These patients will have been treated at or referred to one of the study centers (The Miriam Hospital/Lifespan or Montefiore Medical Center/Albert Einstein College of Medicine. See facilities section Form FDA 1572) for management of relapsing *C. difficile*

infection (CDI). Subjects will be randomized 1:1 to receive true vs. sham intervention. Approximately half of the subjects will receive the proposed intervention (fecal transplant administered through the colonoscope as a suspension of donor stool in saline). The remaining subjects will receive a sham intervention (colonoscopy with infusion of a suspension of their own stool in saline). The estimated duration of this study is 24 months.

No patient will be excluded from recruitment on the basis of ethnicity, race or handicap. Subjects are expected to range in age from 18-74 (age range of patients treated with FMT thus far at the 2 study centers 18-86). Based on our preliminary data, we expect the mean age of the subjects to be 61 years. Subjects will all be outpatients and though some may have co-morbidities (such as diabetes or cardiovascular disease), these conditions will be stable at the time of enrollment (ASA class III or lower). All subjects will be fully informed about the purposes of the study, and risks and benefits of the study, and informed consent for all phases of the study will be obtained. Patients who agree to participate will be free to withdraw from the project at any time.

The intervention, FMT, will be administered directly through the scope at time of colonoscopy. This will be given as a single dose of a 300 cc solution of donor stool in saline. Though-the-scope administration of FMT was chosen based on methods used at the study centers and described in the most recent and largest published case series to date[18,22,23,28-31]. This method has the advantage of allowing one to directly infuse the transplant throughout the entire colon, while also allowing for direct examination of the colonic mucosa; which is often indicated in the recurrent cases to detect any coexistent colonic pathology which may be a factor in the patient's ongoing CDI relapse. These exams will yield important data in this first randomized controlled human trial. Additionally, through-the-scope infusion is generally well tolerated by patients. Transplanting the fecal suspension into the proximal colon enables subjects to retain the material more easily and reliably for an adequate time period compared to infusion via enema and is generally effective after a single infusion.

We are collaborating with investigators at the University of Minnesota, Minneapolis who will perform microbiome analysis on the stools collected from donors and subjects as part of this study. These investigators will also perform studies of metabolism (pre and post FMT) using urine collected from subjects. Stool and urine from donors and subjects will be frozen at -70 Celsius within 6 hours of collection. The samples will be packed in dry ice and shipped regularly overnight to the University of Minnesota research laboratory.

Rationale for including special classes of subjects (children ages 18-21)

Though relapsing CDI is relatively rare in children as compared to adults, our clinical experience using fecal microbiota transplantation has included patients as young as 19. There is no reason to believe that relapsing CDI has different pathophysiology or would respond differently to fecal microbiota transplantation in patients ages 18-21. The study includes children 18-20, but does not include anyone less than 18 years of age (the age of emancipation in Rhode Island and New York).

Rationale for the sample size

Given that FMT will be administered as a single dose on the day of randomization, the relatively short duration of the study, and communication with investigators conducting a clinical trial of fecal transplant in Canada, we anticipate a low dropout rate. Nonetheless, our sample size for recruitment was adjusted up to account for the possibility of an estimated 10% dropout. Even in the absence of a statistically significant difference, the sample size chosen will allow for good initial efficacy data. It will also help determine the feasibility of this study design for a larger multicenter clinical trial.

A. Potential Risks to Subjects

There are 3 areas of risk to subjects associated with participation in the proposed study. These include:

1) Physical risks related to the colonoscopy
2) Theoretical risks (infectious and otherwise) related to FMT and
3) Psychological or other risks related to confidentiality and loss of privacy.

Risks of ingestion of the colon preparation include the risk of dehydration and minor electrolyte imbalances. Standard colonoscopy risks include the risk of bowel perforation, bleeding, and adverse cardiopulmonary events related to sedation. The infusion of the liquid fecal matter will prolong the colonoscopy by less than 5 minutes and adds no additional risk to the colonoscopy. Many adverse effects of colonoscopy resolve shortly after the procedure has been completed, but in some cases abdominal discomfort and gaseous pain side can persist for several hours. There have been no infectious complications directly attributable to FMT reported in the literature to date. However, since the process involves infusion of one person's "body fluids" into another person, transmission of an infectious agent or other disease or condition remains a theoretical possibility. From infusion of donor microflora, they could potentially acquire antibiotic resistance or risk factors for chronic diseases such as diabetes, inflammatory bowel disease, or colon cancer. Risks will be minimized by a rigorous donor-selection process and evaluative studies on stool and blood of the donor to exclude transmission of infectious agents prior to FMT. Donors will not have history of the metabolic syndrome, systemic autoimmunity, chronic pain syndromes, neurologic or neurodegenerative disorders, malignancy, diarrheal disorder or use of antibiotics within 3 months. Subjects will be informed that the treatment or procedure may involve these theoretical risks and additional risks that are currently unforeseeable. A single patient treated in Rhode Island (M, 78) with longstanding, quiescent ulcerative colitis developed moderate inflammatory bowel disease flair 10 days following FMT. He responded readily to prednisone/mesalamine and *C. difficile* did not recur. It is possible that either the CDI or the FMT contributed to flare of his previously stable IBD. Never the less, patients with a history of IBD will be excluded from enrollment in this study. Infusion of fecal material containing a potential agent to which the subject is allergic is also a concern and so patients with a history of severe (anaphylactic) food allergy will be excluded from this study and donors will be queried regarding ingestion of potential allergens during the 5 day period preceding donation.

Physical Risks to Subject:
<u>Very Likely</u>
• Mild to moderate abdominal pain or gaseous discomfort during/after colonoscopy
• Fatigue the day of the colonoscopy from sedatives
• Blood drawing: pain, bruising, feeling faint, slight risk of infection
• *C. difficile* infection recurrence in subjects who receive sham treatment
<u>Less Likely</u>
• Nausea with possible vomiting from ingestion of colon prep solution
<u>Less Likely But Serious</u>

- Contracting infection from donor specimen
- Allergic reaction to unknown antigen present in donor stool
- Risks and side effects related to the colonoscopy including bleeding, bowel perforation and adverse cardiopulmonary events related to sedation
- Acquisition of antibiotic resistance or risk factors for chronic diseases such as diabetes, inflammatory bowel disease, obesity, or colon cancer

B. Potential Risks to Donor
This study also involves individuals (donors) recruited to donate stool for FMT. There are 3 areas of risk to the potential stool donor. These include:
1) Physical risks related to laboratory testing and the stool collection protocol and
2) Psychological risks related to revealing sensitive information during donor screening process
3) Risks related to confidentiality and loss of privacy.
Healthy related and volunteer donors will be recruited at each site. In order to exclude donors at high risk of passing on infection, a donor screening questionnaire (Appendix 1) will be administered. This questionnaire does contain sensitive and potentially embarrassing questions about incarceration, drug use, and high risk sexual behaviors. Additionally, they will be asked questions about their baseline health

status and co-morbidities. Laboratory tests drawn as part of the screening process will include testing for HIV, viral hepatitis and syphilis. These serologic results, if found to be abnormal, could cause psychological distress to the donor. However, the benefit of being made aware of a previously undiagnosed infectious condition outweighs this risk. Potential donors may experience psychological distress if they are excluded from donating stool based on these screenings. To facilitate proper stool consistency, donors will take a single dose of an osmotic laxative on the night prior to donation.
Physical Risks to Donor:
Very Likely
• Blood drawing: pain, bruising, feeling faint, slight risk of infection
Less Likely
• Mild nausea or abdominal discomfort from ingestion the osmotic laxative
Nonphysical Risks to Donor:
Less Likely
• Embarrassment or psychological distress from answering questions regarding drug use and sexual habits asked during the donor screening process.
• Psychological distress (guilt and embarrassment) if screening questionnaire or laboratory test results exclude the person from donation.
Unlikely
• There is also risk of compromising donor privacy through breach of data confidentiality of sensitive protected health information such as results of HIV or viral hepatitis testing.

XI. Adequacy of Protection against Risks

A. Recruitment and informed consent
Participants will be recruited from the Center for Women's Gastrointestinal Health at The Miriam Hospital in Providence, RI and from the Gastroenterology academic practice at Montefiore Medical Center/Albert Einstein College of Medicine in Bronx, NY. After a patient has been referred for relapsing CDI and determined to meet inclusion criteria, the clinician will introduce him/her to the research assistant. Research staff will carefully explain all aspects of the study to a potential recruit, including the risks and benefits and obtain participant's written informed consent. Recruits will be informed of the treatment commitment, amount and general types of assessments, the follow-up telephone interview procedures, and clinic visits. They will be given detailed descriptions of the colonoscopy with FMT procedure. They will be informed of the requirements to have laboratory blood work and to submit stool specimens up to 3 days before, as well as 2 weeks and 8 weeks after the FMT procedure. The research assistant will orally describe the material written in the informed consent document and answer any questions the participant may have. Participants will be reminded that they are not required to participate in the study and that they will receive the standard care provided by their physicians at The Miriam Hospital or Montefiore Medical Center/Albert Einstein College of Medicine regardless of whether or not they choose to participate. Consent documents will be read aloud. Participants who give their consent will sign a copy of the document and will be given a signed copy of the informed consent document.
Subjects may identify a preferred donor (related or unrelated) or chose to receive stool from a healthy volunteer donor (in cases where a known donor is unavailable, unqualified or unwilling). Potential volunteer donors who are healthy will be recruited at each site to serve in a pool through an Institutional Review Board-approved process. Each potential donor will then be contacted by the research staff and asked to participate in the donor consent process. Research staff will carefully explain all aspects of the study to a potential donor, including the risks and benefits and obtain participants' written informed consent. Recruits will be informed of the commitment including a detailed description of the donor screening process and a stool donation protocol. The research staff will explain that a thorough medical history interview, focused physical exam and screening questionnaire will be completed. They will be informed that the questionnaire asks potentially sensitive questions regarding incarceration, drug use, and sexual habits. They will be informed of the requirements to have laboratory blood work (including HIV

testing) and stool studies and to submit stool specimen on the day of the fecal transplant procedure. The research assistant will orally describe the material written in the informed consent document and answer any questions the potential donor may have. Potential donors will be reminded that they are not required to participate in the study. Consent documents will be read aloud. Participants who give their consent will sign a copy of the document and will be given a signed copy of the informed consent document.

B. Protection against risk
Data and safety monitoring will take place to assure the safety of subjects (see section XI. below). All participants will be reminded that their participation is voluntary and that they can withdraw at any time without penalty.
Additionally, the risks described above will be minimized by the following procedures:

1. We will minimize the risk of potential coercion by following standard procedures for obtaining informed consent from both subjects and stool donors. We will begin this process during the intake, where we will clarify the nature of the study and possible alternatives upfront. Prior to enrolling subjects in the research, we will fully explain the study procedures, risks, benefits, and alternatives, emphasizing that the subject's participation has no impact on the other services they receive at The Miriam Hospital/Lifespan (or Montefiore Medical Center/Albert Einstein College of Medicine). Also, subjects who do not consent or who withdraw during the study period will continue to receive appropriate treatment if needed. All subjects and donors will be reminded that there is no penalty for those who choose to not participate or to withdraw from the study and that their decision to participate does not impact the standard services they receive through the hospital. Subjects will not receive financial compensation for their participation. Volunteer donors will be paid a small stipend of $25/stool or blood draw (to cover their time & burden of screening/stool collection).

2. We will minimize potential risks due to loss of confidentiality by having all information collected and handled by research staff trained to deal appropriately with sensitive clinical issues. Potential donors will also be informed about the risk of being ineligible to donate stool due to positive results on screening questionnaires or laboratory testing. Results of donor medical interview, screening and laboratory testing will be kept separate from subject data and will not be available to the subject at any time. All information will be treated as confidential material and will be available only to research staff. All information will be kept in locked file cabinets. Computer data files will be available only to authorized personnel and no names or obvious identifying information will be stored in data files. No participant will be identified in any report of the project. Further, when contacting participants for follow-up, no identifying information other than the first name of the research assistant will be used when leaving messages or speaking to anyone other than the participant him/herself. Written consent will be obtained to contact other persons for the purpose of locating the participant for follow-up and participants can refuse or revoke such consents. No information about participants will be released without their permission or where required by law.

3. We will minimize the theoretical risks of infectious disease or other condition possibly transmitted through FMT by using donor screening protocols modeled after blood banks and organ transplant programs. Potential volunteer donor will undergo thorough screening to determine eligibility for donation. The primary purpose of the donor examination and interview is to ensure that the donor is in good health, and to identify risk factors for diseases transmissible by stool. The donor interview will be used to identify risks for diseases and conditions for which there are no laboratory tests, for which tests are not sensitive enough to detect infectious disease agents, and for which tests are unable to identify early stage or window period infections. Potential donors will be interviewed using a donor screening questionnaire (Appendix item 1) based on the Donor History Questionnaire materials prepared by the AABB Donor History Task Force for use in screening blood donors. Additional questions which are felt relevant to fecal microbiota transplantation including recent use of antimicrobials, and history of

malignancy, gastrointestinal, neurologic or autoimmune disorders have been added to this questionnaire. Donor screening and serologic testing for relevant communicable diseases will be performed based on relevant portions of FDA guidelines for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps). This includes testing donors for HIV types 1 and 2, Hepatitis A, Hepatitis B, Hepatitis C, and *Treponema pallidum.* Donor stool will be tested for bacterial culture, Culture for *Listeria monocytogenes* and *Vibrio* (*parahaemolyticus and cholerae*), Rotavirus via EIA, *Clostridium difficile* toxins A&B, Fecal *Giardia* & *Cryptosporidium* antigens, *Cyclospora*, *Isospora* and Ova and Parasites. Subjects will be tested for HIV 1 & 2, Hepatitis A, B and C and Syphilis to confirm baseline status and prevent future questions about disease transmission. Potential donors will undergo a thorough medical history and examination to exclude any diseases which could (theoretically) be transmitted through the microflora including gastrointestinal diseases (IBD, IBS, chronic diarrhea or constipation), metabolic syndrome, autoimmune or atopic conditions, malignancy, or neurologic/neurodegenerative disease. Donors will be questioned on the day of donation for FMT and those who report fever, vomiting, diarrhea or other symptoms of infection within the last 30 days or ingestion of potential allergen where the recipient has a known allergy to the agent will not be permitted to donate.

4. We will minimize the risk of severe *C. difficile* relapse by maintaining close clinical contact with all subjects. Subjects will be encouraged to contact the clinical team if they experience recurrence of diarrhea, fever or abdominal pain so that stool can be tested for *C. difficile* toxins A & B and antibiotics can be resumed if necessary. Subjects will be contacted via telephone by a study representative 2 and 7 days after the treatment and then biweekly for a total of 8 weeks. They will have follow up visits in the clinic at 2 and 8 weeks post-FMT. We expect 50-60% of sham-treated subjects may relapse with *C. difficile* infection. Relapsing subjects will be treated with an appropriate therapy (typically vancomycin) for the acute infection and will be offered true FMT if they were in the sham group. We do not expect many subjects who receive true FMT to relapse (2-10%). If any of subjects from this arm to relapse, they will be offered a repeat treatment with FMT using stool from an alternate donor.

XII. Safety monitoring protocol

Safety monitoring plan

An external Data and Safety Monitoring Board (DSMB) will be assembled to evaluate the data and safety to subjects enrolled in the study. The DSMB will consist of 3 medical doctors who have experience in clinical trials, with specialization in gastroenterology and/or infectious disease. We do not anticipate any difficulty in recruiting these qualified, independent board members. Initially, the Board will convene with the PIs to review the study protocol and review the guidelines for data and safety monitoring. This will include establishing standard procedures for daily and weekly monitoring by the local internal reviewers (PIs and study personnel). Following this initial meeting, at each quarterly meeting the DSMB will evaluate recruitment, the progress of the trial, subject retention, data quality and confidentiality. The DSMB will review blinded data which will be provided by the unblinded research assistant at each site. Treatment arm received will be provided as key code A or B. If, due to group differences, the DSMB determines access to unblinded data is necessary for safety monitoring reasons, this will be provided to them by the unblinded research assistant at each site. In addition, they will review subjects' clinical status, rates of adverse events and whether or not there have been any changes in risk to participating subjects. This quarterly review will ensure that subject risk does not outweigh study benefits. A report generated from each of these meetings will be retained at the study site and will be forwarded to the hospitals' IRBs, to the FDA (in accordance with Investigational New Drug (IND) regulations and to the NIH. The DSMB will be available to convene outside of the appointed meeting schedule, if necessary, due to concerns regarding a particular subject, or due to any adverse events during the study. The DSMB will make appropriate recommendations for changes in the study protocol, if needed.

Subjects will have a diary card to record solicited adverse events (AEs) and fever for 5-7 days post-transplant and unsolicited AEs for approximately 1 month post-transplant. There will be space on the card to record the intensity and duration of the AEs along with any medications and actions taken. Solicited AEs would be events that might be expected following the administration of study product, which will include (but are not limited to) abdominal pain, bloating, or flatulence. AEs related to the colonoscopy such as rectal bleeding or pain will also be solicited. Solicited AEs will be collected and reviewed at the 2 week visit and unsolicited events will be reviewed and collected at the 8 week visit. Subjects will be contacted via telephone by a study representative 6 months after the last treatment to record any SAEs, new medical conditions/diagnoses or changes in medical conditions/medications since last study contact.

The safety of participants will be monitored during each contact with study participants. Both anticipated and unanticipated adverse events and problems will be formally monitored and recorded. Unanticipated serious adverse events or problems will be reported to the hospital and university IRBs (as per local reporting requirements), the FDA (within 15 days; or 7 days for unexpected fatal or life threatening events) and the NIH. Anticipated and less serious adverse events will be submitted annually in reports to the IRBs, FDA and NIH. The Principal Investigators will be responsible for monitoring the safety and efficacy of this trial, executing the Data and Safety Monitoring (DSMB) plan, and complying with the reporting requirements. The PIs will provide a summary of the DSMB's report to NIH on an annual basis as part of the progress report. The DSMB report will include the participants' sociodemographic characteristics, expected versus actual recruitment rates, retention rates, any quality assurance or regulatory issues that occurred during the past year, summary of adverse events and serious adverse events, and any actions or changes with respect to the protocol. The DSM report to NIH will also include, when available, the results of any efficacy data analysis conducted.

XIII. Data monitoring plan

Data will be collected using standardized paper forms and will only be identified with the study's UIN of the subject. The codes that link the name of the subject and the study UIN will be kept confidential by the Principal Investigators in a secured cabinet. Collected forms will be transported to the PIs' data entry center. Data will be entered in the computer independently by trained data entry staff, and discrepancies will be corrected by the principle investigator, based on source documents. The quality of the data will be monitored once per month. Data quality will be monitored by random inspection of the completed forms by one of the research assistants
and any problems detected will be discussed with the PIs. Descriptive statistics will be computed periodically and reported in aggregate as part of the study monitoring process. The interim analysis will reject the null hypothesis of no difference between groups at a two-sided alpha of 0.3%.

XIV. Educational Training

Since October 1, 2001, Lifespan has required that researchers and IRB members read "Protecting Study Volunteers in Research" (Dunn & Chadwick) and complete the related exam. This process has served as initial certification. In June 2005, The Office of Research Administration contracted with CITI, a Collaborative Institutional (modular) Training Initiative program, for our Human Subjects protection and HIPAA training for all research personnel. Currently this program offers our researchers a basic human subject's protection course as well as a refresher course which we require every three years. Documentation of successful completion is automatically generated and is printed directly by the researcher. The PI will maintain documentation of course completion for all study personnel.

XV. ClinicalTrials.gov Requirements

As required by law, this trial will be registered in ClinicalTrials.gov no later than 21 days after the first subject is enrolled. The reporting of summary results information (including adverse events) will be done no later than 1 year after the study completion date. The principle investigator will be responsible for registering the trial and results reporting.

APPENDIX B. FMT DONOR HISTORY QUESTIONNAIRE

Are you:
1. Feeling healthy and well today? Yes No
2. Currently taking any medication for infection? Yes No
Have you:
3. Taken any antibiotics within the past 6 months? Yes No
4. Had any fevers, vomiting, diarrhea or other symptoms of infection within the past 4 weeks? Yes No
In the past 8 weeks have you
5. Had any vaccinations or other shots? Yes No
6. Had contact with someone who has had the Smallpox vaccine? Yes No
In the past 12 months have you:
7. Had a blood transfusion? Yes No
8. Had a transplant (organ, tissue, bone marrow, dura mater- brain covering)? Yes No
9. Had a skin or bone graft? Yes No
10. Come into contact with someone else's blood? Yes No
11. Had an accidental needle stick? Yes No
12. Had sexual contact with anyone who has HIV/AIDS? Yes No
13. Had sexual contact with a prostitute or anyone else who takes money or drugs as payment for sex? Yes No
14. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything NOT prescribed by their doctor? Yes No
15. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates? Yes No
16. *Female donors*: Had sexual contact with a male who has ever had sexual contact with another male (male donors circle "I am male")? Yes No I am male
17. Had sexual contact with a person who has hepatitis? Yes No
18. Lived with a person who has hepatitis? Yes No
19. Had a tattoo? Yes No
20. Had an ear or body piercing? Yes No
21. Been treated for syphilis or gonorrhea? Yes No
22. Been in lockup, jail or prison for >72 hours? Yes No
In the past three years have you
23. Been outside the United States or Canada? Yes No
List location/time spent:_____
From 1980 through 1996,
24. Did you spend time that adds up to three (3) months or more in the United Kingdom? Yes No
25. Were you a member of the U.S. military, a civilian military employee or a dependent member of the U.S. military? Yes No
From 1980 to the present,
26. Did you spend time that adds up to five (5) or more years in Europe? Yes
27. Receive a blood transfusion in the United Kingdom or France? Yes No
From 1977 to the present, have you
28. Received money, drugs, or other payment for sex? Yes No
29. *Male donors*: had sexual contact with another male, even once (female donors circle "I am female")? Yes No I am female

Have you EVER

30. tested positive for HIV/AIDS virus? Yes No
31. used needles to take drugs or steroids or anything NOT prescribed by your doctor? Yes No
32. used clotting factor concentrates? Yes No
33. had viral hepatitis? Yes No
34. had any type of cancer (including leukemia)? Yes No
35. had sexual contact with anyone who was born or lived in Africa? Yes No
36. been in Africa? Yes No
37. had sex for drugs or money? Yes No
38. had any of the following gastrointestinal diseases or problems?
    - Irritable bowel syndrome? Yes No
    - Crohn's disease? Yes No
    - Ulcerative Colitis? Yes No
    - Chronic diarrhea? Yes No
    - Gastrointestinal cancers? Yes No
    - Celiac disease? Yes No
39. received growth hormone made from human Pituitary glands? Yes No
40. Have any of your relatives had Creutzfeldt-Jakob disease? Yes No

General Medical History

41. Do you have any autoimmune diseases (for example: Rheumatoid arthritis, Multiple Sclerosis, Lupus) Yes No

If yes, please list:_____

42. Do you have any neurologic diseases (for example: Parkinson's, Autism, ALS)? Yes No

If yes, please list: _____

APPENDIX C. COLLECTION AND PREPARATION OF STOOL FOR FMT INFUSION

A. Stool collection

1. Donors and Subjects are supplied a toilet hat and clean, sealable containers for collection and transport of stool. Containers are labeled with the name, date of birth and date/time of stool collection.
2. Collected stool is immediately refrigerated, transferred to the endoscopy facility on ice within a biohazard bag (but not frozen) and used within 6 hours of collection.
3. Immediately prior to scheduled FMT procedure, the non-blinded research assistant (RA) takes possession of the donor and subject stool specimens. These are labeled with UINs of donor and subject.
4. Following randomization, either subject or donor stool material is processed for FMT infusion.

B. Location & preparation of processing area

- Stool is processed in a designated area at each site. This area is not to be accessed by the patient or investigators to avoid compromise of blinding.
- Universal precautions are used during processing (gown, gloves, eye protection).
- Clean counter surface is covered with a Chux® pad.
- After the FMT, all surfaces are wiped with hospital-approved disinfectant solution.

C. Preparation materials

- 1 liter bottle of sterile, non-bacteriostatic normal saline
- Digital scale
- 60 cc disposable slip (catheter)-tip syringes
- Clean gauze
- polypropylene specimen containers

D. Preparation method

1. Processing of stool takes place immediately before scheduled procedure.
2. 500 cc of saline is poured out of the 1 liter bottle and discarded.
3. The research assistant suspends 100 grams of stool in 500 cc of sterile normal saline within the bottle to create a dilute suspension. Should available stool be < 100 grams, the stool is mixed in a reduced volume of saline to approximate the desired dilution.
4. The bottle is closed tightly and shaken vigorously for ~1 minute to homogenize the solution.
5. This solution is then filtered through a sieve to remove larger particulate matter.
6. The fecal suspension is drawn into five 60 cc slip (catheter) tip syringes for infusion.
7. Syringes are delivered to the procedure area for administration during colonoscopy.
8. The remaining stool specimens are further processed and collected in polypropylene specimen containers for storage/shipping and eventual microbiome analysis (see Appendix 4).

APPENDIX D. COLLECTION AND PREPARATION OF STOOL AND URINE FOR STORAGE & SHIPPING

A. Stool Collection: Subjects and Donors are supplied toilet hats and clean, sealable containers for collection and transport of stool. Containers are labeled with name, date of birth and have space to record the date and time of collection. Collected stool is immediately refrigerated, transferred to the study facility on ice within a biohazard bag and used for FMT or processed for storage within 6 hours of collection. Two samples from each stool specimen are banked. One is for shipping to the collaborating investigator for microbiome studies. The other remains at the study facility for internal banking until the conclusion of the study (at least until the 6-month telephone contact with the last study patient
Subjects provide stool specimens at the following time points:

1. Between enrollment and 3 days prior to FMT (for microbiome analysis)
2. On the day of FMT (collected during the bowel purge for possible use in sham FMT)
3. 2 and 8 weeks post FMT (for microbiome analysis)

Donors provide stool specimen on the day of FMT procedure only (for microbiome analysis and possible use in FMT)

B. Urine Collection: Subjects and donors are provided sterile urine specimen containers for collection and transport of urine. The containers are labeled with name, date of birth and have space to record date and time of collection. Collected urine is immediately refrigerated, transferred to the study facility on ice within a biohazard bag and processed for storage within 3 hours of collection. Two samples from each urine specimen are banked. One is for shipping to the collaborating investigator. The other remains at the study facility for internal banking until the conclusion of the study (at least until the 6-month telephone contact with the last study patient
Subjects will provide urine specimens at the following time points:

1. Between enrollment and 3 days prior to FMT
2. 2 and 8 weeks post FMT

Donors provide a urine specimen on the day of FMT procedure only

C. Preparation

1. Stool obtained from subjects and donors is transported on ice to the laboratory and immediately processed (within 6 hours of initial collection). Individual, unprocessed stool samples from subjects and donors is portioned into 5 gram aliquots. These specimens are immediately frozen at -70° Celsius within a designated freezer within the research facility. Freezers are alarmed, with daily temperature logs and generator backup.

2. Urine obtained from subjects and donors is transported on ice to the laboratory and immediately processed (within 3 hours of initial collection). Approximately 25-50 mL of urine is frozen at -70° Celsius within a designated freezer within the research facility. Freezers are alarmed, with daily temperature logs and generator backup.

D. Storage
Specimens are stored for up to 3 months in -70° Celsius freezer within the research laboratories at the study sites (facilities section Form FDA 1572).

E. Shipping:
Specimens are batched quarterly and shipped on dry ice via Federal Express overnight to the laboratory of collaborators at the University of Minnesota, Minneapolis.

APPENDIX E. PROCESSING AND ANALYSIS OF FECAL MICROBIOTA

DNA was extracted from 250-500 mg of fecal samples using the MoBio PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). The V5+V6 regions of the 16S rRNA gene were amplified using the BSF784/1064R primer set,[1,2] along with a no-template-added control, by the University of Minnesota Genomics Center (UMGC; Minneapolis, MN, USA). Amplicons were gel purified and pooled in equal amounts. Paired-end sequencing (2 × 300 nt) was performed on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) by the UMGC. Sequence data were processed, binned to operational taxonomic units, and taxonomic assignments were made against the Ribosomal Database Project using Mothur software ver. 1.34.0[3]. Data are archived in the Sequence Read Archive at the National Center for Biotechnology Information under BioProject accession number SRP066964.

Sequence data were processed and analyzed using Mothur ver. 1.34.0.[3] Sequences were trimmed to 150 nt followed by paired-end joining using the fastq-join program.[4] Joined sequences were quality trimmed using the following criteria: average quality score of 35 over a 50 nt sliding window, no homopolymers ≥8 nt, no more than 2 nt mismatches from primer sequences, and no ambiguous bases.[5] High-quality sequences were aligned against the SILVA database ver. 115[6] and subjected to a 2 % pre-clustering step to further remove probable sequence errors.[7] Chimeric sequences were identified and removed using UCHIME software.[8] Samples were rarefied to 25,000 sequences per sample to reduce bias in comparisons.[9] Clustering of operational taxonomic units (OTUs) was performed at 97% identity using the furthest-neighbor algorithm. Taxonomic classification was performed against the version 14 data release from the Ribosomal Database Project.[10]

Sequence data were processed, binned to operational taxonomic units, and taxonomic assignments were made against the Ribosomal Database Project using Mothur software ver. 1.34.0(11). Processing details are described in supplementary methods. Alpha- and beta-diversity were also evaluated using Mothur ver. 1.34.0. Shannon indices and abundance-based coverage estimate (ACE) parameters were calculated to assess parametric and non-parametric alpha diversity. Differences in beta diversity (community composition) were performed using analysis of similarity (ANOSIM). Variation in OTUs among sample groups was performed using Kruskal-Wallis test.[11] For all community comparisons, donor samples that were not used to treat patients and samples from patients who dropped out of the study were removed from the dataset. ANOVA analysis was performed using XLSTAT ver. 2015.5.01.23039 (Addinsoft, Belmont, MA, USA). All statistics were evaluated at $\alpha = 0.05$.

APPENDIX F. ADVERSE EVENTS (AEs)

"Chills" was reported more frequently in the autologous (placebo)-treated group than in the donor (heterologous) FMT treated group, and this approached significance (P=0.053). The rates of all other solicited AEs (fever, abdominal pain, bloating, nausea, vomiting, diarrhea, flatulence, anorexia and constipation) did not differ significantly between groups. The most common AEs reported in both groups were abdominal pain, fatigue, gas and diarrhea.

Four serious adverse events (SAEs) were reported, though none were directly related to FMT.

- A 76-year-old woman on chronic anticoagulation for a history of deep vein thrombosis was found to have a large adenomatous colon polyp at the time of the study FMT. She underwent piecemeal polypectomy during another colonoscopy 4-months post-donor FMT and was subsequently hospitalized for post-polypectomy bleeding requiring blood transfusion and repeat colonoscopy for hemostasis.

The 3 other SAEs occurred in placebo-treated patients:

- A 35-year-old woman was hospitalized overnight 3-months post-procedure for a self-limited diarrheal illness. Stool was *C. difficile* negative at that time.
- A 37-year-old woman was hospitalized for self-limited diarrhea and vomiting 9-weeks post-procedure and again for exacerbation of an underlying mood disorder 1 month later.
- A 73-year-old woman was hospitalized with severe CDI 3-days after autologous FMT. Symptoms resolved with vancomycin and she was subsequently treated open-label with donor stool has not suffered further CDI recurrence.

Several subjects reported new medical conditions or changes in established medical conditions at the 6-month follow-up phone call.

- One subject reported a 20-pound weight gain after donor-FMT
- One had been diagnosed with a tiny pulmonary nodule requiring follow-up imaging after donor FMT.
- A patient with a history of rectal cancer reported a recurrence after donor FMT
- One had experienced worsening of chronic shoulder pain after donor FMT.
- One reported improvement in "dry mouth" and was able to stop cevimeline after donor FMT.

Two subjects had undergone planned, elective surgery within 6 months of FMT (total knee replacement and laparoscopic cholecystectomy) and been treated with peri-operative antibiotics. No subject reported a late recurrence of CDI.

APPENDIX G. DEVIATIONS FROM PROTOCOL

The treatment arms of New York subjects BS1 and BS2 were unblinded after the 8-week endpoint, rather than at the end of study as planned. Rhode Island subject RS5, who had a previous pattern of late recurrences and who had been treated with donor FMT, complained of feeling unwell at the 8-week follow-up visit, but had not yet developed ≥ 3 watery stools per day required of clinical failures. Symptoms escalated rapidly and her stool was documented to be *C. difficile* PCR positive within 12 hours of reaching the study endpoint. She was classified as a failure and her recurrent CDI resolved after a course of vancomycin followed by open-label FMT using donor stool. New York subject (BS16) did not follow up for the 2- and 8-week clinic visits, but did complete all follow-up telephone contacts and did not develop recurrent diarrheal symptoms after FMT. He was classified as a clinical cure. Thirty-seven subjects received FMT using less than the 100 grams of stool that had been described in the protocol. Since these subjects had discontinued vancomycin, prepped and been randomized, we elected to treat them with the amount of material that was available.

APPENDIX H: ADDITIONAL RESULTS OF MICROBIOTA ANALYSES

Figure 1. Genus-level classification of OTUs that varied significantly between time points by Kruskal-Wallis test ($P < 0.05$) at the Bronx (A) and Rhode Island (B) sites.

Figure 1.A

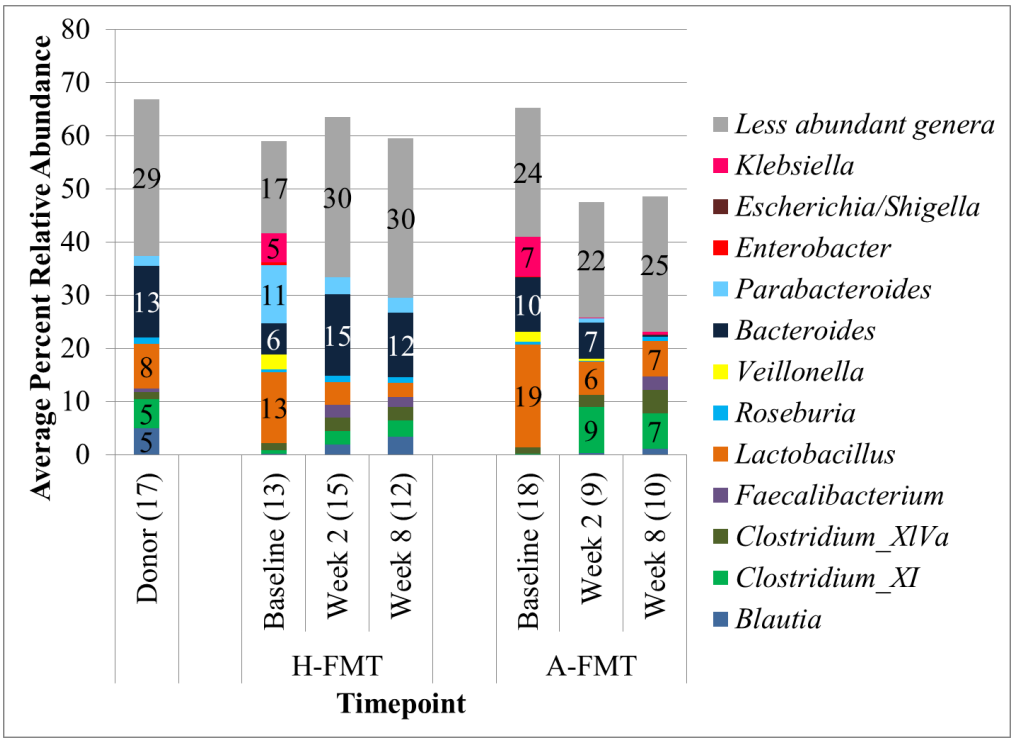


Figure 1.B

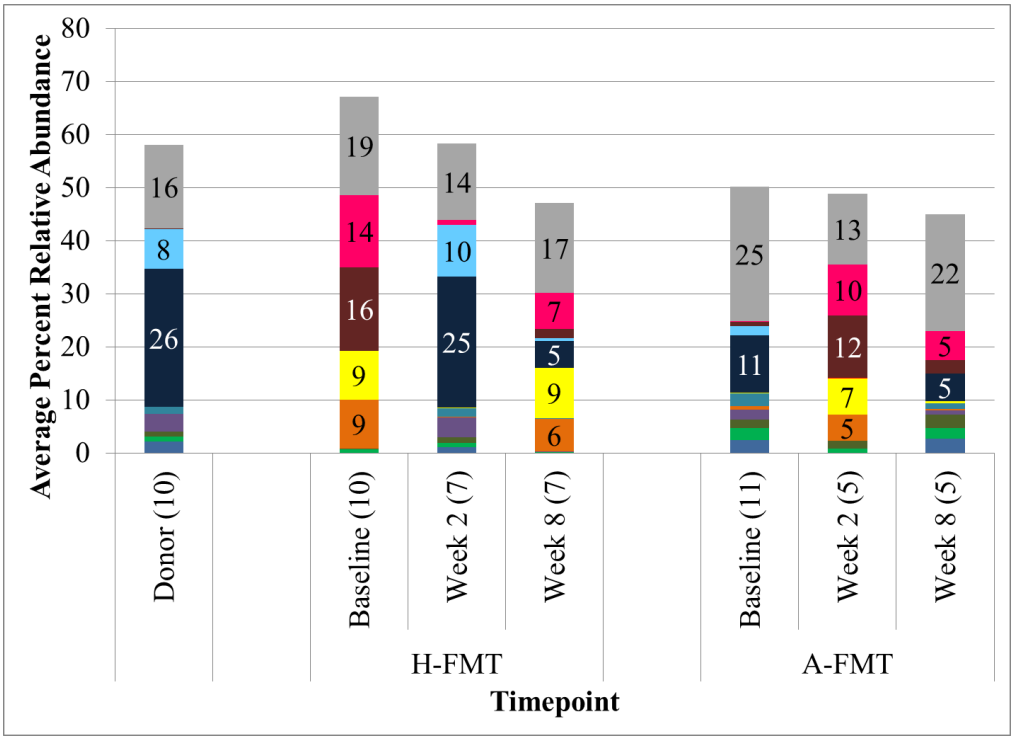


Figure 2. Distribution of phyla in samples from patients at the Bronx (A) and Rhode Island (B) sites. Numbers in parentheses indicate sample size.

Figure 2.A

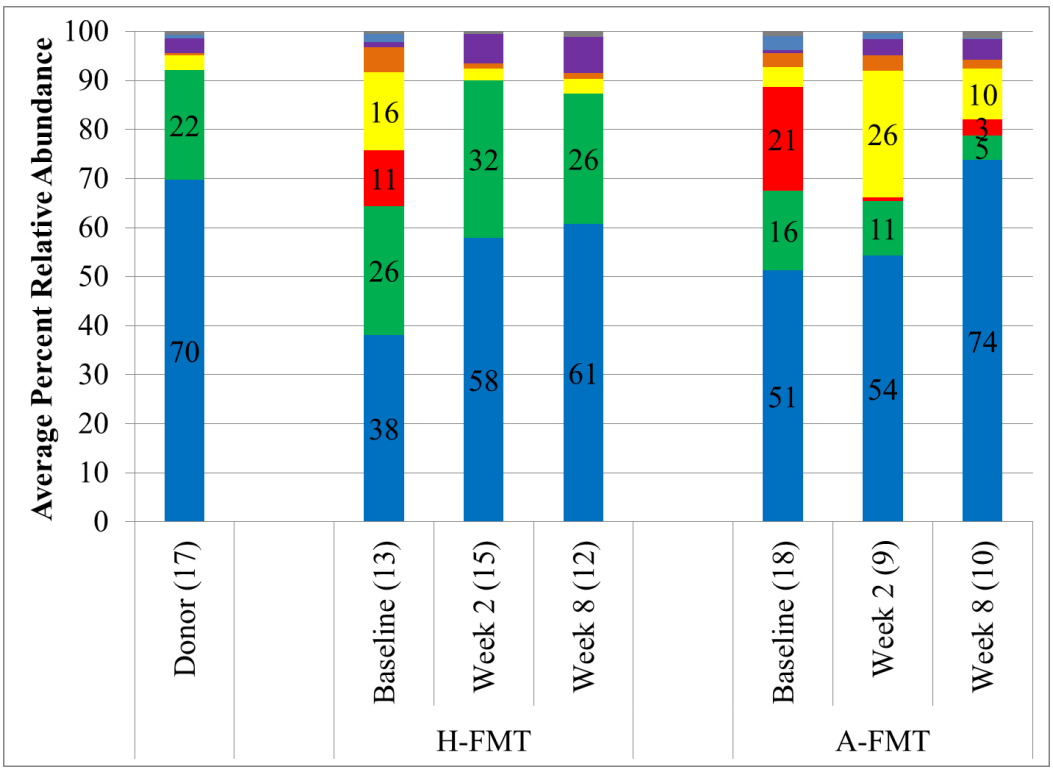


Figure 2.B

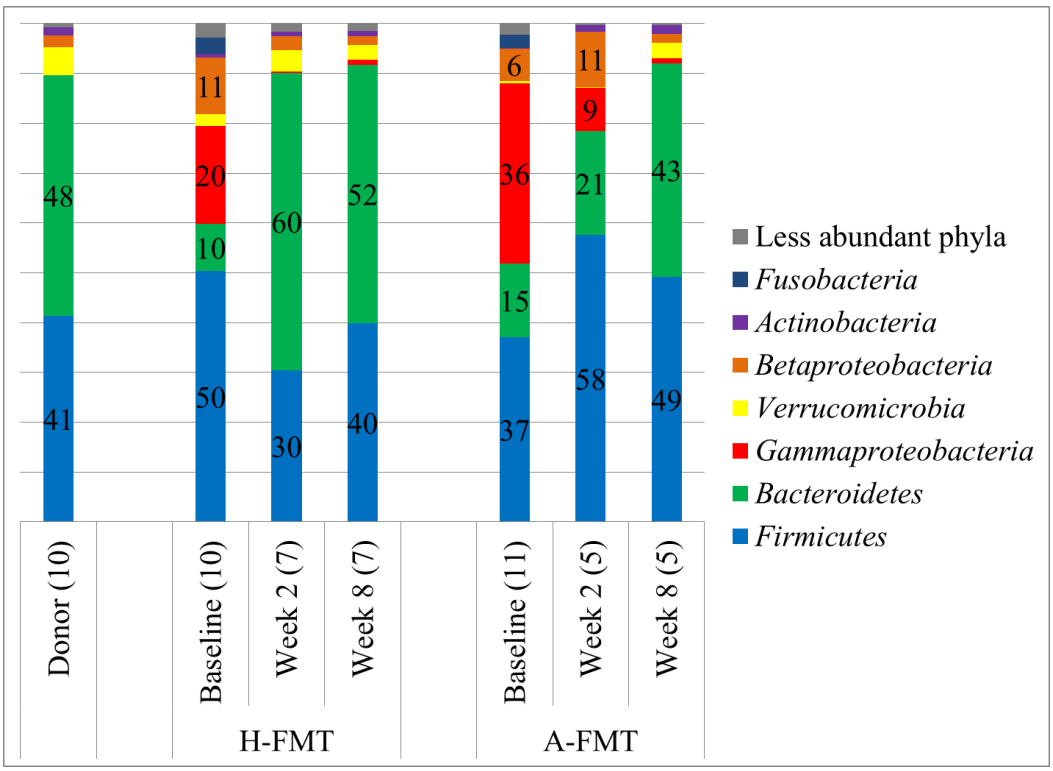


Figure 3. Distribution of phyla in pre-FMT fecal samples at the Bronx and Rhode Island sites with and without prior treatment with fidaxomicin.

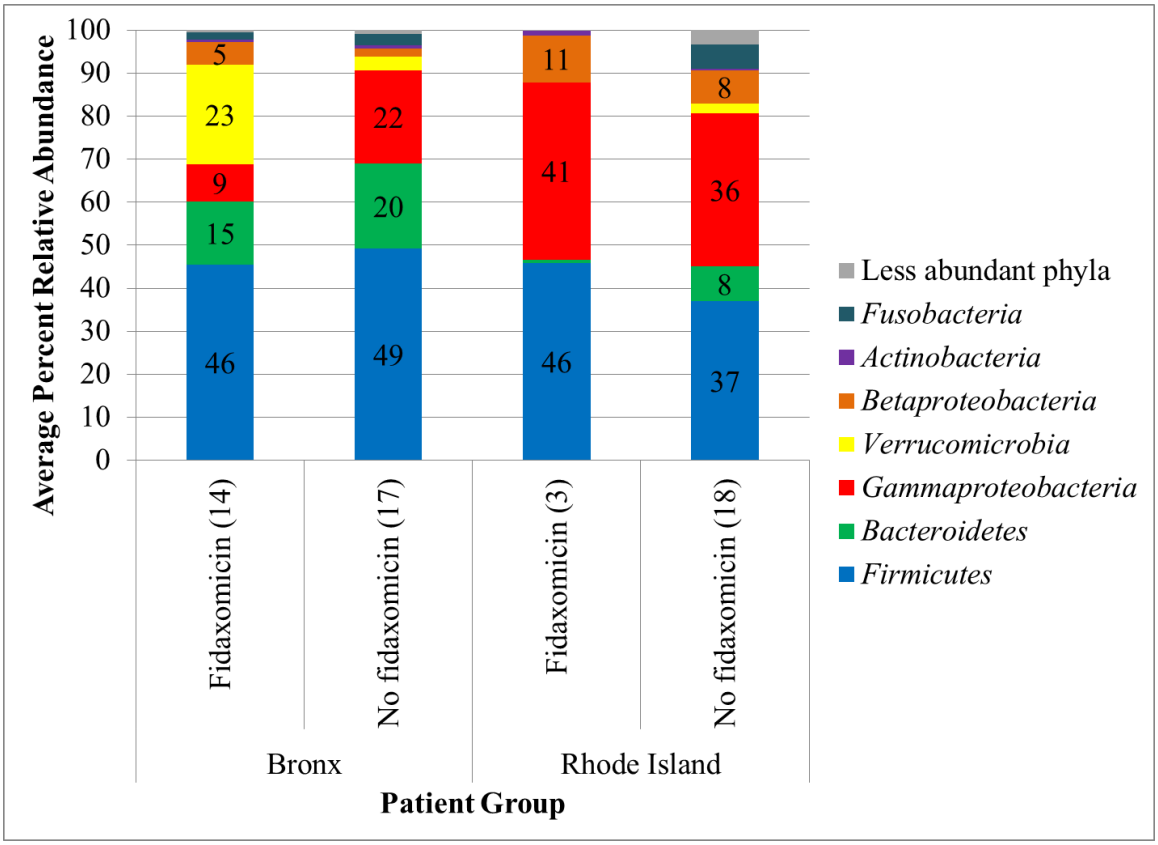


Figure 4. Distribution of phyla in patients who failed to clear *C. difficile* infection at the Bronx site following donor FMT (A) and at the Rhode Island site following placebo FMT with open label FMT follow-up (B)

Figure 4.A

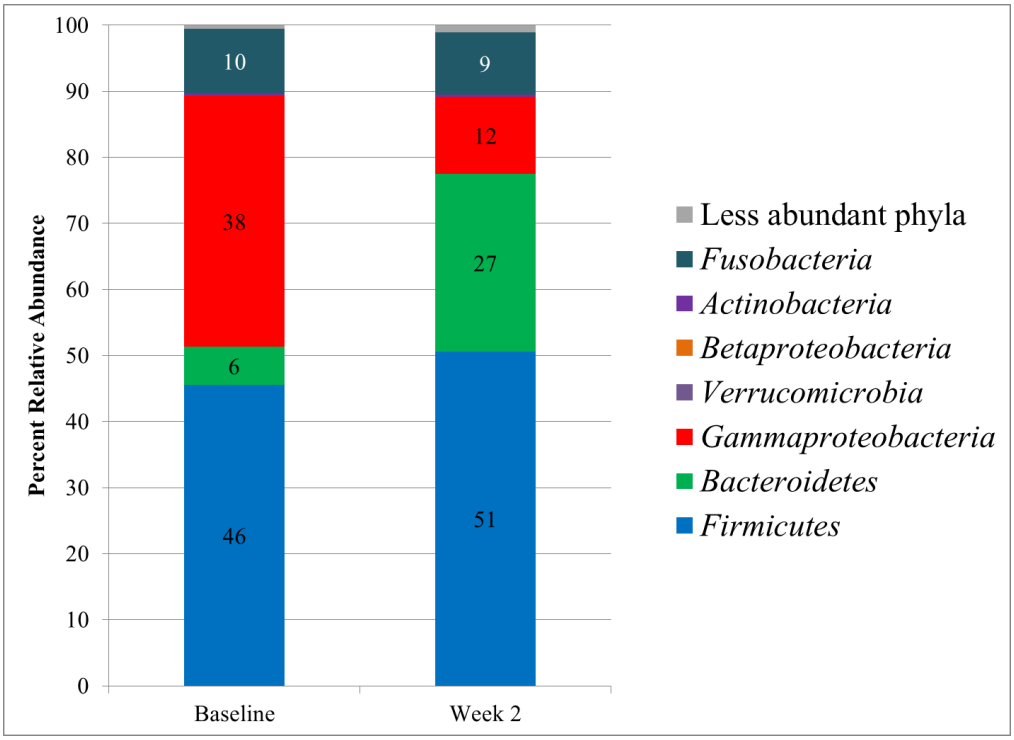


Figure 4.B

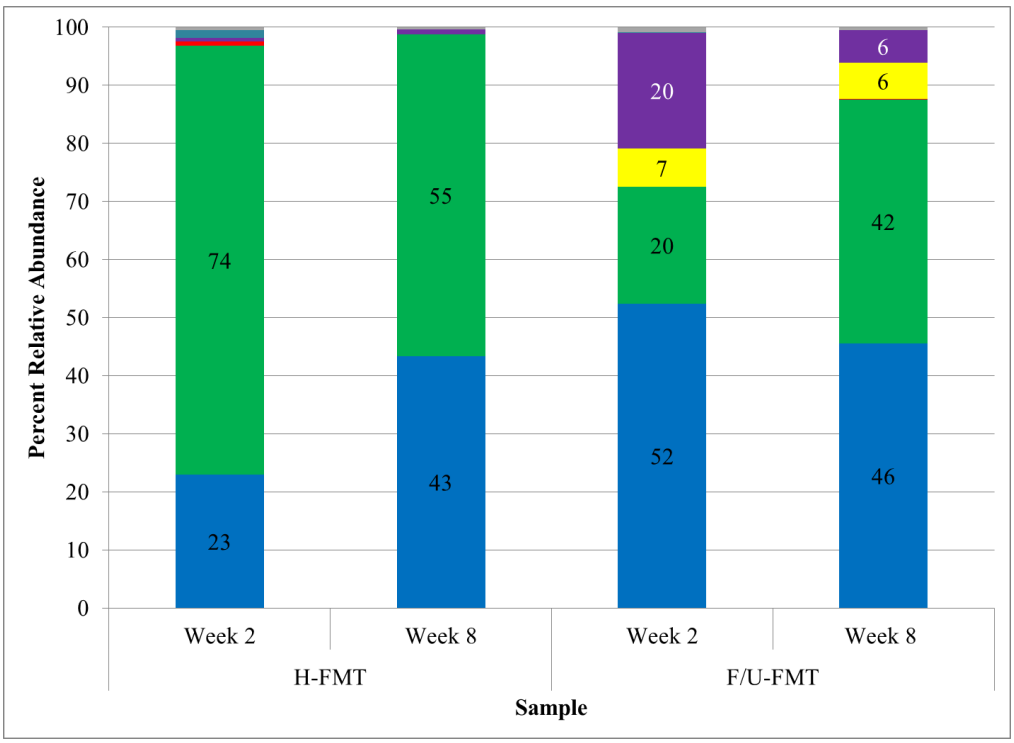


Table 1. Alpha diversity indices (mean ± standard error) for donor, recipient, and sham samples. *Post-hoc* statistics were calculated separately for Bronx and Rhode Island sites.

Location	Sample Group	Time Point	n	Shannon Index	ACE Index
Bronx	**Donor**	**Donor**	**17**	**3.95 ± 0.05[a]**	**1011 ± 24[b,c]**
	Donor Recipient	**Pre-FMT**	**13**	**2.86 ± 0.14[c]**	**627 ± 60[d]**
		Week2	**15**	**3.92 ± 0.09[a]**	**1176 ± 56[a,b]**
		Week8	**12**	**4.01 ± 0.04[a]**	**1397 ± 135[a]**
	Sham	**Pre-FMT**	**18**	**2.90 ± 0.17[c]**	**645 ± 50[d]**
		Week2	**9**	**2.93 ± 0.25[b,c]**	**782 ± 56[c,d]**
		Week8	**10**	**3.59 ± 0.18[a,b]**	**1062 ± 96[a,b,c]**
Rhode Island	**Donor**	**Donor**	**10**	**3.60 ± 0.17[a]**	**1189 ± 178[a]**
	Donor Recipient	**Pre-FMT**	**10**	**2.60 ± 0.20[c]**	**505 ± 59[c]**
		FMT	**9**	**2.59 ± 0.31[b,c]**	**587 ± 94[b,c]**
		Week2	**7**	**3.40 ± 0.25[a,b,c]**	**978 ± 93[a,b,c]**
		Week8	**7**	**3.57 ± 0.15[a,b]**	**1082 ± 80[a,b]**
	Open Label Recipient	**Week2**	**10**	**3.37 ± 0.74[a,b]**	**1087 ± 502[a,b]**
		Week8	**7**	**3.70 ± 0.43[a]**	**1291 ± 433[a]**
	Sham	**Pre-FMT**	**11**	**2.12 ± 0.10[c]**	**431 ± 4[c]**
		FMT	**10**	**2.22 ± 0.19[c]**	**466 ± 38[c]**
		Week2	**5**	**2.99 ± 0.91[a,b,c]**	**967 ± 606[a,b,c]**
		Week8	**5**	**3.00 ± 0.38[a,b]**	**729 ± 118[a,b,c]**

[ab,c,d]Samples sharing the same superscript did not differ significantly at α = 0.05.

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